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Constitutive expression of *MKS1* confers susceptibility to *Botrytis cinerea* infection independent of *PAD3* expression

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Abbreviations: SA, salicylic acid; *B. cinerea*, *Botrytis cinerea*; *Pst* DC3000, *Pseudomonas syringae* pv tomato DC3000; MAPK, mitogen activated protein kinase; R-gene, resistance genes; JA, jasmonate

Signal transduction through MAPK cascades is essential for eukaryotic cell response to various extracellular stimuli, such as the induction of innate immune responses. *Arabidopsis thaliana* relies in particular on three of its 20 MAPKs, MPK3, -4, -6, for a proper immune response. Recently we showed that one MPK4-substrate, MKS1, is required for basal resistance against the virulent *Pseudomonas syringae* and the oomycete *Hyaloperonospora arabidopsidis*. Overexpression of MKS1 (35S-*MKS1*) led to increased resistance to the same pathogens but also to an increased susceptibility toward the fungi *Botrytis cinerea*. MKS1 interacts with the transcription factor WRKY33, which in turn controls the regulation of *PAD3* and *CYP71A13*, two genes, required for proper resistance to *B. cinerea*. Therefore, we tested if the increased susceptibility toward *B. cinerea* from 35S-*MKS1* was due to deregulation of WRKY33 targets. *PAD3* and *CYP71A13* expression is similar in 35S-*MKS1* and WT after *B. cinerea* treatment suggesting another mechanism controls 35S-*MKS1* susceptibility.

MPK4 is part of a signaling cascade that consists of MEKK1 and MKK1/MKK2. Deletion mutants of *mekk1*,[1,2] *mkk1*/*mkk2*,[3] double knockout and *mpk4*,[4] share a dwarf phenotype and MEKK1 and MKK1/MKK2 are required for proper MPK4 activation.[1-3] MKS1 was identified in a yeast-two-hybrid screen for interactors of MPK4 and found to be an MPK4 substrate and in vivo partner.[5] Importantly, deletion of *MKS1* abrogates some of the *mpk4* phenotypes.[6,7]

We recently found that MPK4-MKS1-WRK33 exist in a nuclear localized complex. Activation of MPK4, for example by bacterial elicitors, leads to MKS1 phosphorylation and dissociation of WRKY33 from MPK4 complexes. Subsequently, WRKY33 binds to the promoter of *PAD3* and promote its expression.[7] A recent publication confirmed that WRKY33 binds the *PAD3* promoter.[8] In addition, Mao et al. find that WRKY33 is a substrate of especially MPK6 and to a lower extent MPK3 and MPK4 (most strongly in the absence or MPK3 and MPK6).[8]

Expression of *PAD3* and *CYP71A13* is required for the production of the antimicrobial compound camalexin, necessary for proper defense against *B. cinerea*.[9] Because overexpression of *MKS1* leads to increased susceptibility to *B. cinerea*[6] and MKS1 and WRKY33 interact in planta and are regulated by MAPK activity, it is possible that 35S-*MKS1* susceptibility is caused by deregulation of WRKY33 targets.

We therefore tested the expression of the WRKY33 targets *PAD3* and *CYP71A13* in L*er* (wild type control), 35S-*MKS1*, *mks1* and *mpk4* before and 48 h after infection with *B. cinerea*. Interestingly, there was no difference in the expression of these two genes between 35S-*MKS1* and wild type (**Fig. 1**). In accordance with previously published data, *mpk4* accumulates *PAD3* and *CYP71A13* transcripts even in absence of infection.[5,7]

Since MPK4 is activated within minutes and releases WRKY33 to bind the promoter of *PAD3* within hours and not days, we tested whether a difference in the early response to *B. cinerea* might explain the different susceptibilities. Real time PCR on cDNA from RNA extracted at time points between 2–12 h after *B. cinerea* treatment did not reveal a strong consistent differential response in 35S-*MKS1* compared with L*er* and *mks1* (data not shown).

It is perhaps not surprising that expression of the 35S-*MKS1* construct does not lead to reduced *PAD3* and *CYP71A13*. The model proposed by Qiu et al.[7] would project that excess MKS1 leads to less WRKY33 sequestered in a complex with MPK4. With recent results from Mao et al. one might suspect more WRKY33 to be readily available for MPK3,(4),6 phosphorylation. However, we do not see consistently higher *PAD3*, *CYP71A13* transcript levels upon induction in 35S-*MKS1*. Moreover higher levels of *PAD3*, *CYP71A13* in 35S-*MKS1* would not explain its susceptibility phenotype.

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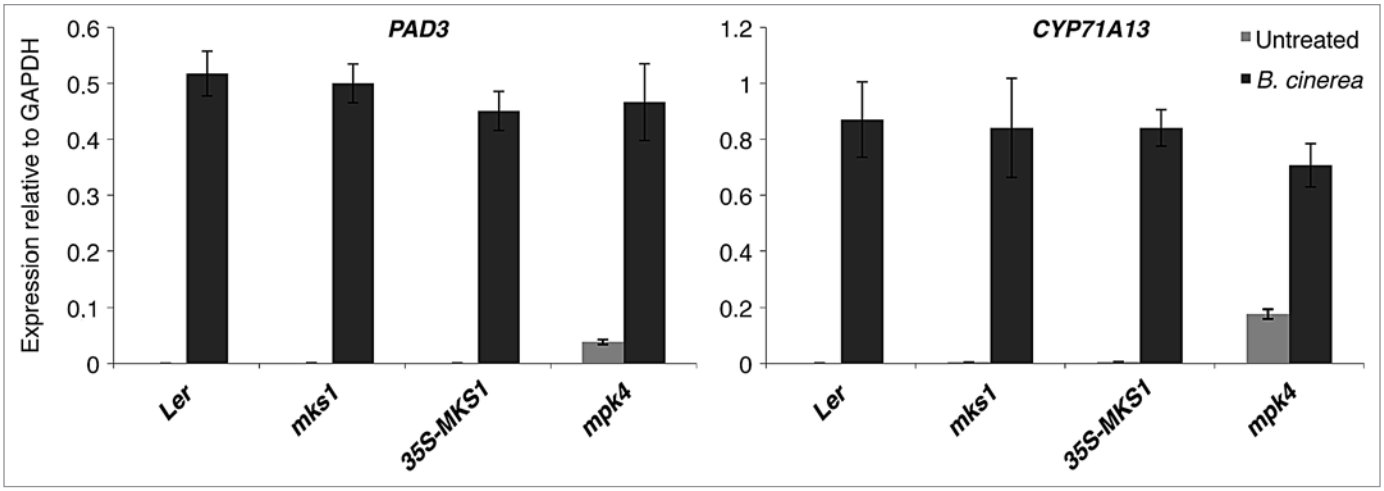

Figure 1. Real-time PCR detection of PAD3 and CYP71A13 on untreated controls or plants treated with *B. cinerea* for 48 h. Samples were tested in triplicate and normalized to GAPDH. Means ± SD is shown.

Like constitutive-defense/lesion-mimic mutants, 35S-*MKS1* plants are semi-dwarfed with occasional lesions, accumulate *PR1* transcript accompanied with increased levels of the plant hormone salicylic acid (SA) and exhibit increased resistance toward *Pseudomonas syringae* pv. tomato DC3000.[5,6]

Although important, camalexin is not the only determinant in *B. cinerea* susceptibility.[10] Other factors include e.g., JA/ethylene signaling.[11] Methyl-JA treatment of 35S-*MKS1* leads to increased levels of the JA marker gene *PDF1.2*.[5] Although *PDF1.2* induction appears to be slightly impaired upon *B. cinerea* infection[6] there is no block in JA/ethylene signaling and this modest decrease is probably not the reason for the enhanced susceptibility.

It has been observed that induction of cell death through the hypersensitive response can facilitate *B. cinerea* infection.[12] This suggests that the lesions sometimes observed in 35S-*MKS1* might be the cause of the enhanced susceptibility and that perhaps the susceptibility is an indirect effect. *Suppressor of salicylic acid insensitive* 2 (*ssi2*) is a lesion-mimic and *B. cinerea*-susceptible mutant. SSI2 encodes a stearoyl-ACP desaturase.[13] Additionally, like 35S-*MKS1, ssi2* is smaller than wild type, accumulates *PR1* transcript and shows enhanced resistance to virulent strains of *Pseudomonas syringae*.[13,14] Surprisingly, *B. cinerea* susceptibility in *ssi2* seems to be independent of its lesions. The double mutants *ssi2*/*pad4* and *ssi2*/*eds5* partially rescue *ssi2* size but still have spontaneous cell death and are much more resistant to *B. cinerea* than the single mutant.[14]

The *ssi2* mutant has low levels of oleic acid (18:1) and its phenotype can be rescued by addition of oleic acid. Recently, it has been shown that the low levels of oleic acid leads to induction of Resistance-gene (R-gene) expression dependent on EDS1 and SA production (through SID2).[15,16] The authors propose that the accumulation of various R-genes and activation thereof is responsible for *ssi2* altered defense-related phenotype.

Whether 35S-*MKS1*-phenotype is due to aberrant R-gene activation remains to be tested. If R-gene activation causes both the 35S-*MKS1* and *ssi2* phenotypes presumably the properties of the specifically activated R-gene(s) result in their secondary phenotypes such as *B. cinerea* susceptibility.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Ichimura K, Casais C, Peck SC, Shinozaki K, Shirasu K. MEKK1 is required for MPK4 activation and regulates tissue-specific and temperature-dependent cell death in Arabidopsis. J Biol Chem 2006; 281:36969-76; PMID:17023433; DOI:10.1074/jbc.M605319200.
2. Nakagami H, Soukupova H, Schikora A, Zarsky V, Hirt H. A Mitogen-activated protein kinase kinase mediates reactive oxygen species homeostasis in Arabidopsis. J Biol Chem 2006; 281:38697-704; PMID:17043356; DOI:10.1074/jbc.M605293200.
3. Qiu JL, Zhou L, Yun BW, Nielsen HB, Fiil BK, Petersen K, et al. Arabidopsis mitogen-activated protein kinase kinases MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4 and MKS1. Plant Physiol 2008; 148:212-22; PMID:18599650; DOI:10.1104/pp.108.120006.
4. Petersen M, Brodersen P, Naested H, Andreasson E, Lindhart U, Johansen B, et al. Arabidopsis map kinase 4 negatively regulates systemic acquired resistance. Cell 2000; 103:1111-20; PMID:11163186; DOI:10.1016/S0092-8674(00)00213-0.
5. Andreasson E, Jenkins T, Brodersen P, Thorgrimsen S, Petersen NH, Zhu S, et al. The MAP kinase substrate MKS1 is a regulator of plant defense responses. EMBO J 2005; 24:2579-89; PMID:15990873; DOI:10.1038/sj.emboj.7600737.
6. Petersen K, Qiu JL, Lutje J, Fiil BK, Hansen S, Mundy J, et al. Arabidopsis MKS1 is involved in basal immunity and requires an intact N-terminal domain for proper function. PLoS One 2010; 5:14364; PMID:21203436; DOI:10.1371/journal.pone.0014364.
7. Qiu JL, Fiil BK, Petersen K, Nielsen HB, Botanga CJ, Thorgrimsen S, et al. Arabidopsis MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. EMBO J 2008; 27:2214-21; PMID:18650934; DOI:10.1038/emboj.2008.147.
8. Mao G, Meng X, Liu Y, Zheng Z, Chen Z, Zhang S. Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in Arabidopsis. Plant Cell 2011; 23:1639-53; PMID:21498677.
9. Thomma BP, Nelissen I, Eggermont K, Broekaert WF. Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. Plant J 1999; 19:163-71; PMID:10476063; DOI:10.1046/j.1365-313X.1999.00513.x.
10. Ferrari S, Plotnikova JM, De Lorenzo G, Ausubel FM. Arabidopsis local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires EDS4 and PAD2, but not SID2, EDS5 or PAD4. Plant J 2003; 35:193-205; PMID:12848825; DOI:10.1046/j.1365-313X.2003.01794.x.
11. Mengiste T, Laluk K, AbuQamar S. Mechanisms of Induced Resistance Against *B. cinerea*. In: Prusky D, Gullino ML, Eds. Postharvest Pathology. Netherlands: Springer 2010; 13-30.

12. Govrin EM, Levine A. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. Curr Biol 2000; 10:751-7; PMID:10898976; DOI:10.1016/S0960-9822(00)00560-1.
13. Kachroo P, Shanklin J, Shah J, Whittle EJ, Klessig DF. A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proc Natl Acad Sci USA 2001; 98:9448-53; PMID:11481500; DOI:10.1073/pnas.151258398.
14. Nandi A, Moeder W, Kachroo P, Klessig DF, Shah J. Arabidopsis ssi2-conferred susceptibility to *Botrytis cinerea* is dependent on EDS5 and PAD4. Mol Plant Microbe Interact 2005; 18:363-70; PMID:15828688; DOI:10.1094/MPMI-18-0363.
15. Chandra-Shekara AC, Venugopal SC, Barman SR, Kachroo A, Kachroo P. Plastidial fatty acid levels regulate resistance gene-dependent defense signaling in Arabidopsis. Proc Natl Acad Sci USA 2007; 104:7277-82; PMID:17431038; DOI:10.1073/pnas.0609259104.
16. Venugopal SC, Jeong RD, Mandal MK, Zhu S, Chandra-Shekara AC, Xia Y, et al. Enhanced disease susceptibility 1 and salicylic acid act redundantly to regulate resistance gene-mediated signaling. PLoS Genet 2009; 5:1000545; PMID:19578402; DOI:10.1371/journal.pgen.1000545.